

Patent Claims

1. A method for the detection of cytosine methylations in DNA is hereby characterized in that

- a) the DNA to be investigated is reacted with a chemical or with an enzyme so that 5-methylcytosine remains unchanged, while unmethylated cytosine is converted to uracil or to another base which differs from cytosine in its base-pairing behavior,
- b) the pretreated DNA is amplified by means of a polymerase and at least one primer, whose 5'-end is joined with a probe via a linker (Scorpion primer),
- c) the primer extension product is separated from the matrix strand,
- d) the probe hybridizes intramolecularly to the primer extension product, whereby the hybridization occurs as a function of the methylation state of the DNA,
- e) a detection is made of whether a hybridization of the probe has occurred.

2. The method according to claim 1, further characterized in that the reaction in step a) is produced with a bisulfite.

3. The method according to claim 1, further characterized in that the reaction in step a) is produced by means of a cytidine deaminase; the unmethylated cytidine reacts more rapidly than methylated cytidine.

4. The method according to at least one of claims 1 to 3, further characterized in that the amplification in step b) is carried out by means of a polymerase chain reaction.

5. The method according to claim 4, further characterized in that the polymerase chain reaction is carried out in the form of the MSP or heavy methyl method.
6. The method according to at least one of claims 1 to 5, further characterized in that the probe bears two signal components which are found in spatial proximity to one another in the inactive form, and which are separated from one another by the hybridization of the probe to a primer extension product.
7. The method according to claim 6, further characterized in that the two signal components involve a quencher-fluorescent dye pair.
8. The method according to at least one of claims 6 to 7, further characterized in that the spatial separation in the inactive form is assured by the secondary structure of the probe, particularly by a hairpin shape.
9. The method according to at least one of claims 1 to 5, further characterized in that the Scorpion primer bears two signal components which are separated from one another in the inactive form, and which are brought into spatial proximity to one another by the hybridization of the probe to a primer extension product.
10. The method according to claim 9, further characterized in that the signal components in the active form generate a signal via fluorescence-resonance energy transfer.

11. The method according to at least one of claims 1 to 5, further characterized in that the probe and another oligonucleotide each bear at least one signal component, whereby the signal components are found in spatial proximity to one another in the inactive form, and are separated from one another by the hybridization of the probe to a primer extension product.

12. The method according to claim 11, further characterized in that the two signal components involve a quencher-fluorescent dye pair.

13. The method according to at least one of claims 11 to 12, further characterized in that the spatial separation between the probe and the other oligonucleotide in the inactive form is assured by a duplex structure.

14. The method according to at least one of claims 1 to 5, further characterized in that the probe and another oligonucleotide each bear at least one signal component, whereby the signal components are separated from one another spatially in the inactive form, and are brought into spatial proximity to one another by the hybridization of the probe to a primer extension product.

15. The method according to claim 14, further characterized in that the signal components in the active form generate a signal via a fluorescence-resonance energy transfer.

16. The method according to at least one of claims 14 to 15, further characterized in that the other oligonucleotide binds in immediate proximity to the probe on the primer extension product.

17. The method according to at least one of claims 1 to 16, further characterized in that several sequences are simultaneously amplified.

18. The method according to at least one of claims 1 to 17, further characterized in that the amplification occurs by means of two Scorpion primers.

19. The method according to claim 18, further characterized in that the Scorpion primers bear different signal components.

20. The method according to at least one of claims 18 to 19, further characterized in that one of the Scorpion primers bears a methylation-specific probe and the other Scorpion primer bears a non-methylation-specific probe.

21. The method according to at least one of claims 1 to 19, further characterized in that one of the Scorpion primers bears a methylation-specific probe and the other Scorpion primer bears a mutation-specific or allele-specific probe.

22. The method according to at least one of claims 1 to 4, further characterized in that a non-methylation-specific PCR amplification takes place, wherein the probe bears a quencher and a dye molecule, which are found in spatial proximity to one another in the inactive form, and which are separated from one another by the hybridization of the probe to a primer extension product ("methyl hairpin").

23. The method according to at least one of claims 1 to 4, further characterized in that an MSP amplification takes place, wherein the probe bears a quencher and a dye molecule which are found in spatial proximity to one another in the inactive form, and which are separated from one another by the hybridization of the probe to a primer extension product ("MSP methyl hairpin").

24. The method according to at least one of claims 1 to 4, further characterized in that a heavy methyl amplification takes place, wherein the probe bears a quencher and a dye molecule which are found in spatial proximity to one another in the inactive form, and which are separated from one another in the hybridization of the probe to a primer extension product ("heavy methyl hairpin").

25. The method according to at least one of claims 1 to 4, further characterized in that a non-methylation-specific amplification takes place, wherein the probe bears a dye molecule and another oligonucleotide bears a quencher which are found in spatial proximity to one another in the inactive form, and which are separated from one another by the hybridization of the probe to a primer extension product ("methyl duplex").

26. The method according to at least one of claims 1 to 4, further characterized in that an MSP amplification takes place, wherein the probe bears a dye molecule and another oligonucleotide bears a quencher which are found in spatial proximity to one another in the inactive form, and which are separated from one another in the hybridization of the probe to a primer extension product ("MSP methyl duplex").

27. The method according to at least one of claims 1 to 4, further characterized in that a heavy methyl amplification takes place, wherein the probe bears a dye molecule and another oligonucleotide bears a quencher which are found in spatial proximity to one another in the inactive form, and which are separated from one another in the hybridization of the probe to a primer extension product ("heavy methyl duplex").

28. The method according to claim 23, further characterized in that the amplification is produced by means of two Scorpion primers, wherein one of the Scorpion primers bears a methylation-specific probe and the other Scorpion primer bears a non-methylation-specific probe ("quantitative methyl hairpin").

29. Use of the method according to at least one of claims 1-28 for the diagnosis or prognosis of cancer disorders or other diseases associated with a change in the cytosine methylation state, for predicting undesired drug interactions, for establishing a specific drug therapy, for monitoring the success of a drug therapy, for the differentiation of cell types or tissues and for the investigation of cell differentiation.

30. Use of Scorpion primers for methylation analysis, particularly for the diagnosis or prognosis of cancer disorders or other diseases associated with a change in the cytosine methylation state, for predicting undesired drug interactions, for establishing a specific drug therapy, for monitoring the success of a drug therapy, for the differentiation of cell types or tissues and for the investigation of cell differentiation.

31. A kit, consisting of at least one Scorpion primer, a polymerase and the necessary reagents for a polymerase chain reaction.